

**Table S1:** IGRs selected for Northern blots.

IGR candidate	IGR length			ORFs		Northern	Detection	Interpretation	Comments
	IGR start	IGR end	(bp)	Flanking genes	orientation				
F2	51518	51775	258	<i>lmo0047 / lmo0048</i>	> >	Not detected			Location of RNAIII in <i>S.aureus</i>
F3	143722	144097	376	<i>lmo0142 / lmo0143</i>	> >	Not detected			
F28	304684	304942	259	<i>lmo0280 / lmo0281</i>	> <	Large bands		Operon	
F10	318375	319049	675	<i>lmo0292 / lmo0293</i>	> >	Not detected			
F13	356323	356567	245	<i>lmo0328 / lmo0329</i>	> >	Large bands		Operon	
F15	<b>513268</b>	<b>514004</b>	<b>737</b>	<i>lmo0476 / lmo0477</i>	< >	<b>≈220 , ≈450 nt</b>	<b>RliA ncRNA</b>		
F16	<b>544137</b>	<b>544923</b>	<b>787</b>	<i>lmo0509 / lmo0510</i>	> >	<b>230, 360 nt</b>	<b>RliB ncRNA</b>		
F17b	677553	678494	942	<i>Ser.tRNA / lmo0638</i>	< >	Large bands		Leader / trailer	
F17	679123	679763	641	<i>lmo0638 / lmo0639</i>	> >	Large bands		Operon	
M2	710749	711121	373	<i>lmo0674 / lmo0675</i>	< >	Not detected			
F23	<b>1154204</b>	<b>1154864</b>	<b>661</b>	<i>lmo1117 / lmo1118</i>	> <	<b>≈220 , ≈280 and 330 nt</b>	<b>RliC ncRNA</b>		
F24	1159830	1160220	391	<i>lmo1124 / lmo1125</i>	> <	Large bands		Operon	
M4	<b>1181036</b>	<b>1181338</b>	<b>303</b>	<i>lmo1150 / 1151</i>	< >	<b>220, 430 nt</b>	<b>RliH ncRNA</b>		<b>antisense <i>lmo1150</i></b>
M5	1276693	1277312	620	<i>lmo1252 / lmo1253</i>	< >	Large bands		Leader / trailer	
F30	<b>1359103</b>	<b>1359352</b>	<b>250</b>	<i>rpsO / pnpA</i>	> <	<b>≈210, ≈300, ≥400 nt</b>	<b>RliD ncRNA</b>	<b>antisense <i>pnpA</i> - Location of <i>sraG</i> in <i>E. coli</i></b>	
F31	1505170	1505312	143	<i>dnaJ / dnaK</i>	< <	Large bands		Operon	Location of tpke11 in <i>E. coli</i>
Ms	<b>1546222</b>	<b>1546608</b>	<b>386</b>	<i>lmo1513 / lmo1514</i>	< <	<b>180 nt</b>	<b>SsrS</b>		
P2	<b>1584638</b>	<b>1584850</b>	<b>213</b>	<i>comC / folC</i>	< >	<b>110 , 135, 225 nt</b>	<b>RliE ncRNA</b>		<b>antisense <i>comC</i></b>
P3b	1639722	1640225	504	<i>rpsD / lmo1590</i>	> <	Large bands		Leader / trailer	
P3	1641004	1641284	281	<i>lmo1597 / tyrS</i>	< <	Large bands		Leader / trailer	
P19	1861090	1861907	818	<i>inIC / rplS</i>	< <	Large bands		Leader / trailer	
Mb	<b>1961644</b>	<b>1962225</b>	<b>581</b>	<i>lmo1887 / lmo1888</i>	< <	<b>385 nt</b>	<b>RnpB</b>		
P7	2038840	2039441	602	<i>lmo1964 / lmo1965</i>	< <	Large bands		Leader / trailer	
P8	<b>2106001</b>	<b>2106329</b>	<b>329</b>	<i>nadA / lmo2026</i>	> <	<b>180, 220 nt</b>	<b>RliF ncRNA</b>		

<b>P14</b>	<b>2386680</b>	<b>2387046</b>	<b>367</b>	<i>lmo2302 / 2303</i>	< <	<b>220, 280 nt</b>	<b>RliG ncRNA</b>
<b>Ma</b>	<b>2509411</b>	<b>2510361</b>	<b>950</b>	<i>lmo2443 / lmo2444</i>	< <	<b>370 nt</b>	<b>SsrA</b>
<b>P16</b>	2706054	2706415	362	<i>rpsJ / lmo2634</i>	< <	Large bands	Leader / trailer
<b>M8</b>	2784107	2784354	248	<i>lmo2710 / lmo2711</i>	< >	Large bands	Leader / trailer
<b>M9</b>	<b>2841896</b>	<b>2842413</b>	<b>518</b>	<i>lmo2760 / 2761</i>	< >	<b>240 nt</b>	<b>RliI ncRNA</b>

Interpretation provided is from Northern blots data using oligonucleotides as probe for ncRNAs (line marked in bold). RliA, C and D lengths were estimated from hybridization performed in low stringency conditions using PCR fragments corresponding to the entire IGRs as probe. “<” and “>” indicate the orientation of ORFs flanking the IGRs.

**Table S2:** Oligonucleotides used in this study

Primer	Sequence (5' to 3')	Position and use	ncRNA
PFF15A	atgcttccttagcaattcc	5' IGR - PCR probe	RliA
PFF15B	ttagctacacttccctgagatatgg	3' IGR - PCR probe	RliA
PFF15D	aacctttcatatagaaccataatcccacg	RliA - Northern; 5'RACE	RliA
PFF15F	gccatttaaccctctaataaataacc	RliA - 5'RACE	RliA
Ig-58F	gaaagtgtaaaatgggtgg	5' IGR - PCR probe - IGR cloning	RliB
PFF16B	gtattattcagttacccccc	3' IGR - PCR probe - IGR cloning	RliB
PFF16D	gctgtcccttgacaaaaatcgcatatcac	RliB - Northern; 5'RACE	RliB
PFF16E	cgctacatgctgtgtgc	RliB - 5'RACE	RliB
PFF23A	cgacggatgaatgattcgtcg	5' IGR - PCR probe	RliC
PFF23B	gggatatctaaaataaaaggaaatggac	3' IGR - PCR probe	RliC
PFF23E	cataacaaagtgtataaatacac	RliC - Northern; 5'RACE	RliC
PFF23F	ggagtttacactctatcccacacagaagac	RliC - 5'RACE	RliC
PFF30A	gagtattaactataactaaatggaaaacac	5' IGR - PCR probe	RliD
PFF30B	tatTTTactctccTTgtttagg	3' IGR - PCR probe	RliD
PFF30C	gctatTTTtagcaggTTTggattcatgtgtgcTTtagacaatc	RliD - Northern; 5'RACE	RliD
PFF30E	gacaatctaaagtgtgcgt	RliD - 5'RACE	RliD
PFP2A	caccccttagtgtaaaaac	5' IGR - PCR probe	RliE
PFP2B	gaataaaatcgagttactgtgag	3' IGR - PCR probe	RliE
PFP2D	ggatTTcagaagataaaatataaaaaacacctg	RliE - Northern; 5'RACE	RliE
PFP2E	cacctgtttccactaatg	RliE - 5'RACE	RliE
PFP8A	gcaacaactgtttcgTTtagctc	5' IGR - PCR probe	RliF
PFP8B	ggcaaccttagTTTatttaaggag	3' IGR - PCR probe	RliF
PFP8C	gcaactttcacaaccaattaatccaccctag	RliF - Northern; 5'RACE	RliF
PFP8E	caccctagctatacattg	RliF - 5'RACE	RliF
IG219F	gcaacaccTTTattt	5' IGR - PCR probe	RliG
IG219R	agcaggagattgttatgtt	3' IGR - PCR probe	RliG
PFP14C	ccagttatggataggatatggatggaaacagaagtgtcgcatc	RliG - Northern; 5'RACE	RliG
PFP14E	cgtcatctgtgagactagtgtt	RliG - 5'RACE	RliG
MV4D	gccatccagtaatgtatggTTggcattctttatg	RliH - Northern	RliH
MV4F	gcgattataatatgtataactaagaacg	RliH - 5'RACE	RliH
MV4H	gctataactaaagaacgacag	RliH - 5'RACE	RliH
MV9C	gtataatactaatttagactatacaaaccgattgcgtgac	RliI - Northern; 5'RACE	RliI
MV9E	gcgtgactccatgttctc	RliI - 5'RACE	RliI
MV9surA	ccaaggaagcatggcaaaatgttacgt	5' IGR - IGR cloning	RliI
MV9surB	ggtaaatagatTTTgtcTTgggttcg	3' IGR - IGR cloning	RliI
FR.PM.rnpB	ccgaagacttagtcatccatcgccgtcaaaccg	Northern	RnpB
FR.PM.ssrA	gcacggaggatcagctatgttatttagg	Northern	SsrA

FR.PM.ssrS	ggttcaaaaatcaggaactatacgaaatacattagg	Northern	SsrS
<b>PF.1035A</b>	cccaactcaagggaaattgttgc	lmo1035 - PCR probe	
<b>PF.1035B</b>	gattaactgtatccaagtccaaacatg	lmo1035 - PCR probe	
<b>PF.1036A</b>	ccttcagatactgttggagtgc	lmo1036 - PCR probe	
<b>PF.1036B</b>	ccttcatttatccaaacggctc	lmo1036 - PCR probe	
<b>PF.2104.A</b>	ctgctgtcggtaaaaagttcgcatctcg	lmo2104 - PCR probe	
<b>PF.2104.B</b>	gctgtccaccataatggcgcaagcatcac	lmo2104 - PCR probe	
<b>PF.2105.A</b>	gttagaaactggagcggagtaccgttgc	lmo2105 - PCR probe	
<b>PF.2105.B</b>	gtgcgccgaactcaaggagctgaatcg	lmo2105 - PCR probe	
<b>FR.T7.F16.K</b>	taatacgactcactatagggagatgtcttttgttaaagcacatcaagcatgtac	T7-synthesis of RliB/gel-shift	
<b>FR.F16.N</b>	gggtgtttagtgatgttggacttttgttgg	T7-synthesis of RliB/gel-shift	
<b>FR.T7.2104.III</b>	taatacgactcactatagggagactgtcggtaaaaagttcgcatctcg	T7-synthesis of lmo2104/gel-shift	
<b>FR.2104.D</b>	atattgccttttgttccaaatgtacaggaccac	T7-synthesis of lmo2104/gel-shift	
<b>FR.T7.P2.K</b>	taatacgactcactatagggagaaatcatctgtcaccccttagttaaaaacagaatagcagagacg	T7-synthesis of RliE/gel-shift	
<b>FR.P2.K</b>	ttagctatgtgcaaaaaaaaattcgctcgctattctg	T7-synthesis of RliE/gel-shift	
<b>FR.T7.0945.X</b>	taatacgactcactatagggagattgcgccttgcattctactaagagagcag	T7-synthesis of lmo0945/gel-shift	
<b>FR.0945.Y</b>	gtcatcttctccctctgtctcttagtag	T7-synthesis of lmo0945/gel-shift	
<b>FR.T7.comE.X</b>	taatacgactcactatagggagaagctatcaaaaaggatttgtcctgtcatggcaatag	T7-synthesis of comEA/gel-shift	
<b>FR.comE.Y</b>	caccatccatataccgtcctaataactattcgccatgcag	T7-synthesis of comEA/gel-shift	
<b>FR.T7.comF.X</b>	taatacgactcactatagggagatttcgttgcataaaaaaaaagaaaaagtgtataactaagggag	T7-synthesis of comFA/gel-shift	
<b>FR.comF.Y</b>	gtccatctgttaccctcccttagttatcac	T7-synthesis of comFA/gel-shift	
<b>FR.T7.M9.L</b>	taatacgactcactatagggagagctaaattttgtatattgtccgtcaattttccg	T7-synthesis of RliI/gel-shift	
<b>FR.M9.K</b>	ggcagacttcgccaatgtaaactgcataaccggaaaaattgcacg	T7-synthesis of RliI/gel-shift	
<b>FR.T7.1035.C</b>	taatacgactcactatagggagacactcgccatgttattacagtaaacctggaaaacacgtagac	T7-synthesis of lmo1035/gel-shift	
<b>FR.1035.D</b>	tttgctctaaaatctttggttatgtctacgtgtttccaag	T7-synthesis of lmo1035/gel-shift	
<b>PMH1</b>	gaagtgtatgcatacaggaaagcc	Deletion hfq	
<b>PMH2</b>	cataatttccctctccaaatctc	Deletion hfq	
<b>PMH3</b>	gagattggagagggaaattatgcctgtgcggataaaggcacg	Deletion hfq	
<b>PMH4</b>	cagcagccaaatattgcgcac	Deletion hfq	
<b>FP.prfA1</b>	gttattcaatcaacgagtaatccgttaaacc	Deletion prfA	
<b>FP.prfA2</b>	atcaaaaacagtattctcaatgagaaactg	Deletion prfA	
<b>FP.prfA3</b>	gaggaatactgtttgtatgtctcatcccccaatcgttttatcg	Deletion prfA	
<b>FP.prfA4</b>	acactactcccaactgacacgcgc	Deletion prfA	
<b>FR.sig1</b>	gacaaaattacattacaactcctgcc	Deletion sigB	
<b>FR.sig2</b>	caccaacaattgttggcacagcaaatgc	Deletion sigB	
<b>FR.sig3</b>	gcatttgctgtccacaattgttggtgcagaggcgttgcagaatgaggaatgt	Deletion sigB	
<b>FR.sig4</b>	ccaaccataaaaatgttaccactgttgg	Deletion sigB	

Mutations by in frame deletion in *hfq*, *prfA* and *sigB* genes were constructed by PCR-ligation. The amplicon obtained was cloned into the pMAD suicide vector (Arnaud *et al.*, 2004) and the recombinant vector was introduced into EGD-e wild type strain (BUG1600, Glaser *et al.*, 2001). Chromosomal deletions are from the translation start codon, nt 4 to 219, for *hfq*; nt 1 to 714 for *prfA*; nt 249 to 715 for *sigB*. For RNA duplex formation, RNAs were synthesized via T7 transcription using PCR fragments as template.

**Table S3:** Parameters used for target predictions.

A-T A-T 9	G-T A-T 13
A-T C-G 22	G-T C-G 25
A-T G-C 21	G-T G-C 21
A-T G-T 6	G-T G-T 5
A-T T-A 11	G-T T-A 14
A-T T-G 14	G-T T-G -13
C-G A-T 21	T-A A-T 13
C-G C-G 33	T-A C-G 24
C-G G-C 34	T-A G-C 21
C-G G-T 14	T-A G-T 10
C-G T-A 21	T-A T-A 9
C-G T-G 21	T-A T-G 13
G-C A-T 24	T-G A-T 10
G-C C-G 34	T-G C-G 15
G-C G-C 33	T-G G-C 14
G-C G-T 15	T-G G-T -3
G-C T-A 22	T-G T-A 6
G-C T-G 25	T-G T-G 5

A-T followed by A-T contributes a positive score 9; A-T followed by C-G contributes a positive score 22, etc.,. These values are the thermodynamic energies of RNA pairings, changed of sign, multiplied by 10 and rounded to the closest integer, to speed up the calculation of alignments. As for bulges and internal loops, the cost of their opening is 8 while for their extension the cost is 5. An additional cost 8 is added for opening and/or closing with the thermodynamically less stable A-T or T-A pairings; G-T or T-G matches at opening or closure of internal loops and bulges are not allowed. For computation reasons, the maximum bulge or internal loop length is fixed at 6.